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Anti-diabetic activity of novel androstane derivatives from *Syzygium cuminii* Linn.

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Abstract

Objective: To study antidiabetic activity of two novel androstane derivatives (JB1 and JB2) isolated from the ethanolic extract of seeds of *Syzygium cuminii* Linn. Materials and methods: Compounds JB1 and JB2 were isolated from ethanolic extract of seeds of *S. cuminii* by normal phase column chromatography using toluene: ethyl acetate (80:20) as mobile phase and their structure was elucidated by spectroscopic methods. Antidiabetic activity of JB1 and JB2 and the ethanolic extract was evaluated using alloxan as diabetogenic agent. The potency of JB1 and JB2 (100 mg/kg) and the ethanolic extract (200 mg/kg), was compared with glibenclamide (5 mg/kg). The elevated blood glucose levels were monitored colorimetrically using glucose oxidase enzyme system. Results: Compound JB1 was found to be 3, 15-dihydroxy Δ^3 androstene [16, 17-C] (6' methyl, 2'- 1, 3-dihydroxy-1-propene) 4H pyran whereas JB2 to be 3-hydroxy androstane [16, 17-C] (6' methyl, 2'-1-hydroxy-isopropene-1-yl) 4, 5, 6 H pyran. Ethanolic extract of seeds of *S. cuminii* reduced elevated blood glucose levels in alloxan-induced diabetes in rats on single dose as well as prolonged treatment. Compound JB1 was found to be more effective than JB2 as it showed peak activity at the end of day three, which was comparable to ethanolic extract.

Key words: *Syzygium cuminii* Linn., Anti-diabetic activity, Glibenclamide, Alloxan

1. Introduction

Syzygium cuminii (Linn) Skeel (Family: Myrtaceae) is known as Jaman or Jam in Hindi and Jambolan in English. It is distributed throughout India and bears fruits that are ellipsoid or oblong, up to 2.5 cm long, black with pinkish juicy pulp; seeds single and shaped like fruit [1, 2]. Seeds of *S. cuminii* are reported to possess antidiabetic [3-5], anti-inflammatory, antipyretic [6-8] and are reported to contain tannins, tannin glycosides, flavonoids, flavonoid glycosides [6]. The seed powder was found to be effective against increased blood glucose levels in experimental animals [9-11] and diabetic human adults [12], the ethanol and hot water extracts were also reported to posses similar effect in experimental animals [13-15]. Further, the seed extracts had showed to decrease in free radical formation out of lipid peroxidation in alloxan induced diabetic rats [16] and the water extract was showed to increase in the activity of hexokinase and decrease in the activity of glucose 6-phosphatase in liver of alloxan dosed diabetic rats [17].

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Literature review on *S. cuminii* revealed that it is an important member of antidiabetic herbs and the seeds are official in The Ayurvedic Pharmacopoeia of India [1]. In the present study two novel androstane derivatives JB1 and JB2 were isolated from the ethanolic extract of seeds of *S. cuminii.* Since the seeds were reported to posses antidiabetic activity and the compounds JB1 and JB2 are present as major phytoconstituents as indicated from the yields (JB1=1.6% and JB2=7.3% with respect to dried extract), they were screened for any possible antidiabetic activity using glibenclamide as standard against alloxan induced diabetes in albino rats. Their potency was compared with that of ethanolic extract.

2. Materials and methods

2.1 Plant material

Fresh fruits of *S. cuminii* were collected from the trees in the neighboring areas of Vallabh Vidyanagar town in the month of June. The outer pulp of the fruits was removed and the seeds were washed with tap water followed by distilled water. The plant material was authenticated and a voucher specimen was submitted to the Department of Pharmacognosy, A. R. College and G. H. Patel Institute of Pharmacy, Vallabh Vidyanagar.

2.2 Isolation and characterization of JB1 and JB2

The seeds were shade dried for two weeks. The seed material was made into moderately course powder. The powder was then dried in oven at 50°C for one hour. The powdered material (3.0 kg) was defatted with petroleum ether (40-60°C) followed by extraction with ethanol (95%) by cold maceration. The solvent was evaporated under reduced pressure. A portion of alcoholic extract was chromatographed in normal phase using 125 cm column (ID 3.5 cm), Silica gel (BDH) and toluene: ethyl acetate (80:20) as mobile phase and the elution was maintained at 30-33 drops/min and 60 fractions of 20 ml each were collected.

The elute of the column was tested by TLC on precoated plates of Silica gel $60F_{254}$ (E. Merk) using toluene: ethyl acetate (80:20) as mobile phase. The developed plates were observed under UV lamp at 366 nm. Some earlier fractions gave single spot with orange fluorescence at R_f 0.75 of JB2 and some later fractions gave blue fluorescence spot at R_f 0.38 of JB1. The fractions which gave single spot at similar R_f were combined and filtered. The filtrate was evaporated under reduced pressure to obtain separated component.

Elemental analysis for isolated compounds JB1 and JB2 was carried out using CHNS/O analyzer, (2400 Series, Perkin Elmer USA) and spectral analysis was done by UV spectroscopy (UV/ Vis double beam spectrophotometer, Lambda 19 of Perkin Elmer, USA) at SICART, Vallabh Vidyanagar. FTIR, (Perkin Elmer, 16PC), 1H-NMR (Brucker, DPX200, Version 3), and EIMS (Shimadzu, QP 508) was done at Sun Pharm Advanced Research Center, Vadodara.

2.3 Preparation of test solutions

JB1, JB2 and alcoholic extract of *S. cuminii* were formulated as suspension in distilled water using Tween 80 as suspending agent, since Tween 80 has a negligible effect on Blood Glucose Level (BGL). The strength of the suspension was according to the dose administered and expressed as weight on dried basis.

2.4 Preparation of standard drug

Glibenclamide was used as the reference drug for evaluating the antidiabetic activity. Daonil tablets (Aventis Pharmaceuticals, Mumbai) containing glibenclamide (2.5mg) were powdered and made into suspension in distilled water using Tween 80 as suspending agent.

The strength of suspension was adjusted to 0.5 mg/ml of glibenclamide and vehicle was prepared without the drug.

2.5 Animals

Wistar strain albino rats of both sex weighing 170-200g were chosen for the screening of the antidiabetic activity. The animals were housed and acclimatized under standard laboratory conditions (temperature: 21-24°C, relative humidity: 40-60% and 12 hr. light-dark cycle) and were allowed on a standard palleted diet (M/s. Pranav Agro Industries Ltd. Vadodara) and water *ad libitum*. 120 mg/kg of alloxan monohydrate (BDH) in normal saline was used orally to induce hyperglycemia [18-20].

2.6 Antidiabetic evaluation

The acclimatized animals were kept for fasting for 24 h with water *ad libitum* and injected intraperitoneally a dose of 120 mg/kg of alloxan monohydrate in normal saline. After one hour, the animals were given the feed *ad libitum*. A 5% dextrose solution was given for a day to overcome the early hypoglycemic phase [21]. The BGL was monitored before alloxanisation and every 24 h after alloxanisation by withdrawing blood from tail by tail tipping method [22]. The BGL was measured colorimetrically using "Glucose enzyme reagent system" manufactured by Span Diagnostics Ltd., Surat. The system uses glucose oxidase method of estimating glucose in the blood [23].

Animals were considered diabetic when the BGL rose beyond 150mg/100ml of blood. This condition was observed at the end of 72 h after alloxanisation. The animals were segregated into five groups of six rats each, taking into consideration the diabetic BGL. Group 1 served as control, group 2 received glibenclamide (5mg/kg, P.O.), group 3 received alcoholic extract (200mg/kg, P.O) and group 4 and 5 received JB1 and JB2 (100mg/kg, P.O.) respectively. The vehicle, standard and test solutions were administered orally, every 24 h for period of seven days, to rats using rubber catheter. Institutional Animal Ethical Committee approved the experimental protocols. The BGL was monitored after 1, 3, 5 and 7 h of administration of the first dose (for acute study) and at the end of 1, 3, 5 and 7 days for prolonged treatment.

2.7 Statistical analysis

To determine the statistical significance of the change in BGL, data obtained was subjected to one way analysis of variance (ANOVA) followed by Dunnet's *t* test. The results are expressed as ± SE, where n=6.

Table 1. Effect of JB1, JB2 and ethanolic extract of *S. cuminii* on BGL of diabetic rats after single oral dose.

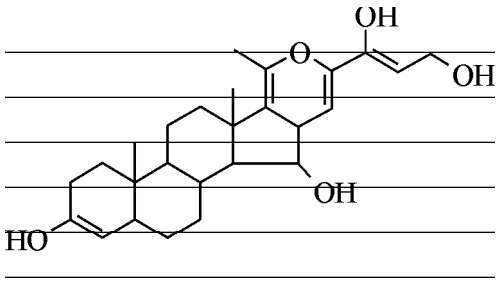
Drug	BGL (mg/100 ml)				
	Initial	**1 hr**	**3 hrs**	**5 hrs**	**7 hrs**
Control	273.5 ± 2.34	279.1 ± 3.25	281.9 ± 3.79	285.7 ± 3.21	291.7 ± 2.39
GLI	274.9 ± 6.7	138.6 ± 0.59*	120.2 ± 1.54**	85.9 ± 0.94**	82.5 ± 1.34**
Eth. Ext.	247.6 ± 4.83	132.1 ± 3.41*	119.8 ± 4.3**	90.6 ± 1.40**	86.5 ± 1.25**
JB1	243.5 ± 4.83	248.3 ± 1.62	240.6 ± 3.82	238.4 ± 3.65	240.2 ± 2.78
JB2	240.6 ± 3.34	245.8 ± 2.30	241.7 ± 1.28	240.2 ± 2.41	235.3 ± 1.86

Values are mean of ± S.E (n=6); *p < 0.05, **p < 0.01 (One-way ANOVA followed by Dunnett's test); BGL: Blood Glucose Level; GLI: Glibenclamide

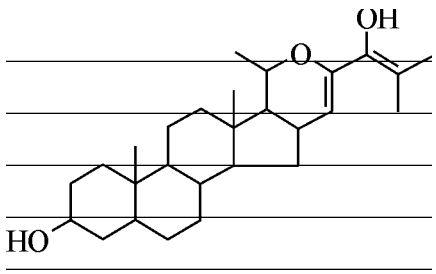
Table 2. Effect of JB1, JB2 and ethanolic extract of *S. cuminii* on BGL of diabetic rats on prolonged treatment.

Drug	BGL (mg/100 ml)				
	Initial	**1 day**	**3 days**	**5 days**	**7 days**
Control	273.5 ± 2.34	298.1 ± 3.25	301.9 ± 3.79	325.8 ± 3.21	332.7 ± 2.39
GLI	274.9 ± 6.72	80.1 ± 0.12**	80.7 ± 1.87**	77.8 ± 1.82**	73.7 ± 1.83**
Eth. Ext.	247.6 ± 4.83	95.2 ± 2.56**	94.0 ± 3.71**	90. 7 ± 1.06**	86.2 ± 1.13**
JB1	243.5 ± 4.83	136.5 ± 2.65*	101.8 ± 2.11**	95.3 ± 2.14**	94.2 ± 1.84**
JB2	240.6 ± 3.34	185.8 ± 3.42*	157.5 ± 2.56*	140.7 ± 3.69*	132.3 ± 4.21*

Values are mean of ± S.E (n=6); *p < 0.05, **p < 0.01 (One-way ANOVA followed by Dunnett's test); BGL: Blood Glucose Level; GLI: Glibenclamide



JB1



JB2

3. Results

Isolated compound JB1 gave pale yellowish colored dry amorphous powder, yield; 1.6%, m.p.; 129-131°C, Elemental analysis, calculated for $C_{26}H_{36}O_5$,: Calcd. C, 72.87; H, 8.47; O, 18.67. Found C, 72.00; H, 9.42; O, 18.58. UV (MeOH): $\lambda_{1(max)}$ = 212nm, λ_2 = 289; FTIR (KBr): $v_{max,}$ cm^{-1} = 3416 (-OH), 2929, 1454 (-CH2), 1673, 803 (C=C), 1169 (C-O-C, cyclic); ^{1}H-NMR, (400 MHz, $CDCl_3$): δ_{ppm} = 0.9 (*q*, 3H, -CH_3, C_{18}), 1.10 (*s*, 3H, -CH_3, C_{19}), 1.25 (*s*, 14H, -CH-, cyclic), 5.3 (*s*, 2H, -C=C-); EIMS: *m/z* (rel. int.) = 428 (M^+, 11.23), 285(9.15), 217(12.02), 135(16.10), 122(18.21), 109(21.02), 94(24.11), 69(35.64), 57(23.87), 55(48.65), 43(100).

Compound JB2 gave pale yellowish brown colored dry amorphous powder, yield; 7.3%, m.p.; 122-124°C, Elemental analysis, calculated for $C_{27}H_{42}O_3$,: Calcd. C, 78.20; H, 10.21; O, 11.58. Found C, 77.54; H, 10.81; O, 11.58. UV (MeOH): $\lambda_{1(max)}$ = 207nm, λ_2 = 285; FTIR (KBr): $v_{max,}$ cm^{-1} = 3416 (-OH), 2922, 1455 (-CH_2), 1674, 738 (C=C), 1108 (C-O-C, cyclic); ^{1}H-NMR, (400 MHz, $CDCl_3$): δ_{ppm} = 0.9-1.0 (*q*, 3H, -CH_3, C_{18}), 1.10 (*s*, 3H, -CH_3, C_{19}), 1.25 (*s*, 16H, -CH-, cyclic), 5.32 (*s*, 2H, -C=C-); EIMS: *m/z* (rel. int.) = 414 (M^+, 28.4), 396(12.6), 329(14.57), 303(10.31), 273(9.42), 248(27.95), 219(9.33), 107(25.63), 95(45.12), 85(48.54), 71(52.87), 56(75.11), 43(100).

The results of evaluation of antidiabetic activity was expressed as the change in BGL and were presented in table 1 and 2. It is clear from table 1 that, after a single dose the standard glibenclamide and ethanolic extract of seeds of *S. cuminii* showed significant reduction in elevated blood glucose levels ($p<0.05$) after 1 h of treatment and the effect reached its peak in 5 h ($p<0.01$). Compounds JB1 and JB2 did not show any decrease in the elevated blood glucose levels on administration of a single dose. In table 2 it can be seen that, on prolonged treatment, glibenclamide and ethanolic extract maintained the antidiabetic activity up to the seven days. JB1 showed significant reduction in blood glucose level ($p<0.05$) at the end of day one. The activity further enhanced and reached its peak by the end of day five and showed a significant fall in the blood glucose level ($p<0.01$) which was almost similar to that of standard and ethanolic extract. Though JB2 also showed significant fall after day one ($p<0.05$) the effect was not as potent as that of JB1.

4. Discussion

Structure elucidation of JB1 and JB2 suggest that they are androstane derivatives with a fused 16, 17-C pyran ring. JB1 is 3, 15-dihydroxy Δ^3 androstene [16, 17-C] (6' methyl, 2'- 1, 3-dihydroxy-1-propene) 4H pyran whereas JB2 is 3-hydroxy androstane [16, 17-C] (6' methyl, 2'-1-hydroxy-isopropene-1-yl) 4, 5, 6 H pyran.

Diabetes mellitus is known to be accompanied by oxidative stress, i.e. with the preponderance of oxidative reactions over the antioxidative protection of tissues [24] accompanied by increased production of reactive forms of oxygen and lipoperoxides.

Precise diabetogenic mechanism of alloxan is not been fully understood yet. Evidences indicate that the alloxan induces pancreatic cell damage which is mediated through the generation of cytotoxic oxygen free radicals [25-27]. The primary target of reactive oxygen species produced from alloxan was proposed to be attacking the DNA of pancreatic cells causing the fragmentation of the DNA [28, 29].

Ethanolic extracts of seeds of *S. cuminii* were shown to reverse the pancreatic cell damage caused by alloxan [13, 30]. The present study reveals that ethanolic extract of seeds of *S. cuminii* reduces the elevated blood glucose levels in alloxan-induced diabetes in rats. The effect was visible within three hours of administration and was similar to the standard glibenclamide. Compound JB1 and JB2 did not show antidiabetic activity after single dose however the effect was visible at the end of day one. JB1 was found to be more effective than JB2 as it showed peak activity at the end of day three, which was comparable to ethanolic extract. The alleviation of diabetes in the animals can be attributed to decrease cytotoxic oxygen free radical formation induced by alloxan.

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References

1. *The Ayurvedic Pharmacopoeia of India*, (2000) Part I, Vol. II, I Edn., Ministry of Health and Family Welfare, New Delhi: 54.
2. *The Wealth of India*, Raw Materials (1989) Vo. X, CSIR New Delhi: 100.
3. Purohit A, Daradka HMM. (2000) *Hamdard Medicus* 43(4): 33.
4. Singh N, Tyagi SD, Agarwal SC. (1991) *Biosphere* 3(1): 29.
5. Gujral MI, Choudhury NK, Srivastava RS. (1954) *Indian Med. Gaz.* 89: 141.
6. Ross IA. (1999) *Medicinal Plants of the World*, Humana Press: New Jersey; 283.
7. Choudhuri ANK, Pal S, Gomes A, Bhattacharya S. (1990) *Phytother. Res.* 4(1): 5.
8. Muruganandan S, Srinivasan K, Chandra S, Tandan SK, Lal J, Raviprakash V. (2001) *Fitoterapia* 72 (4): 369.
9. Al-Zaid M, Hassan MAM, Badir N, Gumaa KA. (1991) *Int. J. Pharmacog.* 29(2): 81.
10. Mueller-Oerlinghausen B, Ngamwathana W, Kanchanapee P. (1971) *J. Med. Assoc. Thailand* 54: 105.
11. Sridhar SB, Sheetal UD, Pai MRSM, Shastri MS. (2005) *Braz J Med Biol Res.* 38(3): 463.
12. Bhatt HV, Gupta OP, Gupta PS. (1983) *Asian Med. J*, 26(7): 489.
13. Sigogneau-jagodzinski MP, Bibal-Prot M, Chanez PB, Ratsimamanga R. (1967) *CR Acad. Sci. Ser. D*, 264(8), , 1119-1123.
14. Chakraborty T, Poddar G. (1984) *J. Inst. Chem. (India)* 56(1): 20.
15. Shorti DS, Kelkar M, Deshmukh VK, Aiman R. (1963) *Indian J. Med. Res.* 51(3): 464.
16. Prince PS, Menon VP, Pari L. (1998) *J. Ethnopharmacol*. 61(1): 1.
17. Prince PS, Menon VP, Pari L. (1997) *Phytotherapy Res.* 11(7): 529.
18. Satyavathi GV, Raina MK, Sharma M. (1976) *Medicinal Plants of India*, ICMR, New Delhi: 461.
19. Rerup CC. (1970) *Pharmacol. Rev*. 22(4): 485.
20. Lukens FDW. (1948) *Physiol. Rev*. 28: 304.
21. Goldner MG, Gomori G. (1944) *Endocrinology* 35: 241.
22. Armin J, Grant RT, Wright RH. (1960) *J. Physiol*. 151: 131.
23. Huggett ASG, Nixon DA. (1957) *Lancet* 2:368.
24. Sinclair AJ. (1993) *Diabet. Rev*. 2: 7.
25. Cohen G, Heikkila RE. (1974) *J. Biol. Chem*. 49:2447.
26. Yamamoto H, Uchigata Y, Okamoto H. (1981) *Biochem. Biophy. Res. Commun.* 103: 1014.
27. Takasu N, Asawa T, Komiya I, Nagasawa Y, Yamada T.(1991) *J. Biol. Chem.* 266: 2112.
28. Okamoto H. (1985) *BioEssay* 2: 15.
29. Hye WR, Ji NL, Hyung RK, Byung HP, Jin WP. (2000) *Exp. Mol. Medicine* 32(1): 12.
30. Sharma SB, Nasir A, Prabhu KM, Murthy PS, Dev G. (2003) *J. Ethnopharmacol*.85: 201.